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Humidity and Rest Period Effects on Viscid Silk Recovery

Catherine Donley

University of Akron Main Campus, cmd110@uakron.edu

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Humidity and Rest Period Effects on Viscid Silk Recovery

Catherine Donley

Department of Polymer Science (9871:499)

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INTRODUCTION

Spider silk is a versatile material. Orb-weaving spiders, spiders that spin what people generally visualize when they think of a two-dimensional spider web, tend to spin seven different silks; and each is unique.¹ An orb weaver's flagelliform silk is quite extensible, more so than its other silk types,² and is used in combination with aggregate silk, a type of aqueous silk glue, to construct the capture spiral of an orb web.^{2,3} The capture spiral engages in energy dissipation by converting work of extension into heat via irreversible deformation.^{4,5,6} Viscid silk (the flagelliform and aggregate silk combination) also has self-healing properties and is able to repair, at least in part, irreversible deformation incurred during extension, if given time during which stress is removed.^{7,8,9} (There is some disagreement about whether or not a full recovery can be made this way. Ashton and Stewart have stated that processing is necessary for spider web silks in general to make a complete recovery.¹⁰ De Tommasi et. al. have stated that spider silks in general can accomplish full recovery via simple rest at higher temperature based on the work of Denny.^{8,7} However, Denny's paper only recorded observing full recovery at elevated temperature for major ampullate silk.⁷ Denny suggested a similar recovering capability in viscid silk based on other observations, but temperature-dependence was not specifically tested for viscid silk.⁷)

Structural design is involved in flagelliform silk's mechanical properties. The proteins of flagelliform silk are believed to incorporate β -spirals, composed of blocks of the repeating peptide GPGGX where X is A, S, V, or Y about 90 % of the time, held together by hydrogen bonds and other intramolecular forces, within their structures.^{2,3,11} Figure 1 shows one of these repeating peptides.

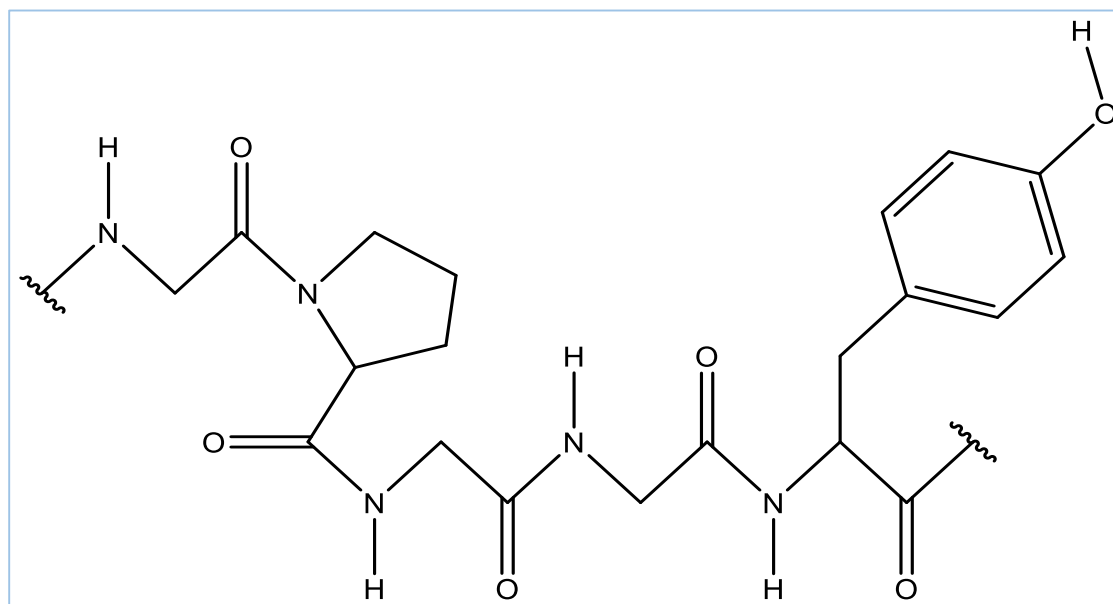


Figure 1. Primary structure of a GPGGX peptide, with Y as the variable amino acid, X. Chains of these peptides create β -spirals.

These proposed β -spirals are believed to serve as springs within the silk structures.¹¹ As the silk extends, hydrogen bonds break, dissipating energy and releasing previously folded up portions of the microscopic silk structure.⁸ Librations within the β -spirals are damped as the molecules extend, causing

a decrease in entropy.^{9,12} Thus, when the load is removed, the silk molecules recoil, according to the librational entropy mechanism, in order to increase their entropy.^{9,12} The hydrogen bonds have opportunity to reform after recoil, repairing, at least partially, the deformation incurred during extension.^{13,8} (In order to regain the silk's original condition, the original hydrogen bonds must reform. If hydrogen bonds form between different sections of the silk molecules, then the silk has not completely recovered. The closer the molecules come to full recoil before hydrogen bond reformation, the more "healing" those hydrogen bonds are. It should also be noted that a return to original condition means that any energy that was dissipated via irreversible deformation has been returned.)

However, water is also known to contribute significantly to the silk's properties. The presence of moisture plasticizes the flagelliform silk, increasing its extensibility.^{2,11} In fact, it has been proposed that water is the dominant factor in flagelliform silk extensibility.¹¹ Water is believed to weaken hydrogen bonds between and within β -spirals, providing more freedom of movement.^{11,9} Water aids in the silk's healing process, an effect also believed to be related to hydrogen bonding, because reformation of hydrogen bonds post-stretch is believed to reduce the silk's self-healing capabilities.^{9,14} (Again, the closer the silk molecules come to full recoil before hydrogen bond reformation, the more "healing" those hydrogen bonds are. Conversely, the farther from complete recoil the silk molecules are before hydrogen bond reformation, the more damaging those hydrogen bonds are.) In fact, complete immersion followed by drying is known to heal any present irreversible deformation in flagelliform silk.¹⁴

For native flagelliform silk, moisture is provided apart from any additional process by the aggregate silk coating.^{2,15} The aggregate silk itself contains glycoproteins, inorganic salts, and multiple hygroscopic organic compounds.^{15,5} Its water content depends on the humidity of the environment.^{16,2}

The goal of this procedure is to investigate the self-healing properties of viscid silk between loading cycles, apart from any external process and with respect to humidity and rest period length, so as to increase its practicality and investigate a trait which would be of use in synthetic materials. Spider silk applications are limited by a lack of complete recovery once irreversibly deformed upon stretching.⁶ Of what use is a structural material if its properties are not constant?⁶ Of what use is a material in a cyclical process if that material must be repaired after each cycle in order to return it to its original quality? It seems then, that the self-healing properties in spider silk and their maximization may be of value. Ultimately, because the use of spider silk is also limited by the small amount attainable,¹⁸ self-healing properties in synthetic materials are likely to be of greater value, particularly because in general, composite materials are of limited use due to their susceptibility to weakening because of repeated damage.¹⁷ However, these properties must first be studied and understood within creation before they can be mimicked by man.^{19,17}

Because water is known to completely heal flagelliform silk and the amount of water around the flagelliform silk within the capture spiral is determined by the humidity of the environment due to the aggregate silk coating, humidity effects on recovery were investigated.^{14,16,2} (This too is the reason viscid silk was studied rather than major ampullate silk, which is also fairly easy to obtain and has self-healing capabilities but lacks an outer, aqueous, humidity-responsive layer.^{7,20}) An indication of how time relates to recovery was also desired. Thus, the effects of rest period length on recovery were investigated as well. However, temperature, extension rate, and maximum extension (following from the fact that loading history influences silk properties) also influence the behavior of spider silk and had to be considered.^{8,4,6} Thus, in cyclical loading tests, humidity and recovery time between cycles were

varied; but all other variables were held as constant as possible. Tests were performed at low, intermediate, and high humidity. Besides a rest period of 5 minutes, which was used by Denny with good results, tests were also performed with virtually no rest period and an intermediate rest period.⁷ The work of extension and hysteresis for successive cycles were analyzed as an indication of how close the recovered silk had come to regaining its initial properties, and thus achieving full recovery. The extensibility of the silk at different humidities was also determined as an indication of how far the silk samples ought to have been stretched in the cyclical tests, and effective diameters of the samples were calculated from diameters of single silk strands (each viscid silk sample from the capture spiral was actually a thread pair) as measured under a microscope.

Results were expected to show that work of extension and hysteresis both decrease over repeated cycles, but most notably between the first and second cycles.⁷ Greater recovery was expected for longer rest periods between cycles and also for greater water content within the aggregate silk at higher humidity. To avoid confusion, full recovery is here defined as a successive cycle's return to the initial properties of the first cycle, rather than 0 hysteresis for any particular cycle. Properties of successive cycles in relation to each other, and not the properties of a single cycle, are in view.

RESULTS AND DISCUSSION

GENERAL

Temperature was between 19.0 °C and 21.0 °C at all times. Though humidity may have risen or dropped steeply and/or fluctuated during the adjustment period, it stayed within about $\pm 6\%$ of the desired value during the tests, though fluctuation sometimes occurred.

TENSILE TESTS

For tensile tests, error in the reading for the load on the specimen, as taken before the tests, was generally in the range of several tens of microNewtons or less. Table 1 contains the averages and standard deviations of true and engineering extensibility at different humidities for silks from the 3 spiders used in hysteresis tests. Maximum strain values for hysteresis tests were chosen based on this data, with the desire to prevent silk breakage (at least for most samples), and still induce enough resistance to be accurately measured during cyclical loading.

	20% Humidity	50% Humidity	80% Humidity

True Extensibility			
Average	0.763	1.132	1.136
Standard Deviation	0.117	0.11	0.165
Engineering Extensibility			
Average	1.160	2.121	2.157
Standard Deviation	0.2	0.3	0.6

Table 1: Average and standard deviation of true and engineering extensibilities at different humidities.

Silk samples from other spiders were also used in tensile tests. Data for individual samples and results incorporating data from other spiders can be found in Appendix II in Tables S1 and S2, respectively.

CYCLICAL TESTS

During cyclical tests, several general observations and/or adjustments were made as detailed here. Firstly, an extension rate of 1 mm/s, was approximately the fastest rate the machine was capable of while still stopping and starting where desired. Tests were performed with this extension rate in order to come as close as possible to the natural extension rate caused by the impact of an insect. (Denny has assembled a list of several insects, all of which, it should be noted, have a maximum speed that is orders of magnitude greater than this extension rate.)⁷ Secondly, samples tested at 50% and 80% humidity were extended to 80% true strain, a value which generated enough resistance in the sample to be adequately measured by the equipment used. However, a significant number of samples tested at 20% humidity were unable to extend to 80% true strain without breaking. As a result, samples tested at 20% humidity were only extended to 60% true strain. Had the samples tested at 20% humidity been capable of the higher strain, greater damage and less recovery would have been expected than that which was observed. This had to be taken into account when searching for humidity-related trends, but was considered to have no bearing on trends related to rest period length, which were determined by comparing data only from tests conducted at the same humidity. Thirdly, of the 54 samples used in cyclical tests (18 per humidity setting), 8 broke during the first cycle before reaching the maximum strain, despite the adjustment in maximum strain just described: 4 samples at 20% humidity, 1 sample at 50% humidity, and 3 samples at 80% humidity. Another sample broke during the fourth cycle at 20% humidity. Fourthly, it was observed that silk samples tested cyclically at 20% humidity became slack as the machine returned to the original sample length, showing the inability of the silk to recover as well at 20% humidity, even at a lower strain, as at 50% or 80% humidity. Trends in recovery were further examined by analysis of changes in work of extension and hysteresis as detailed below.

Greater recovery was evidenced by a smaller change in work of extension. Graphs of work of extension averages and standard deviations at the 3 different humidities can be found in Appendix II in Figures S1-S3. Comparisons were difficult to make using these figures, since the considerable variation among silk samples caused the graphs to have different starting points and large standard deviations. Thus, for the sake of comparison, work of extension for a cycle in any particular test was calculated as a percentage of the work of extension for cycle 1 of that test. Figures 2-4 show these graphs for rest periods of 2 seconds, 1 minute, and 5 minutes, respectively, with each figure containing values for the 3

humidity settings. Figures 5-7 show these graphs for 20%, 50%, and 80% humidity, respectively, with each figure containing values for the 3 rest periods. The P-values for comparisons between work of extension data for a particular cycle can be found in Appendix II in Table S3.

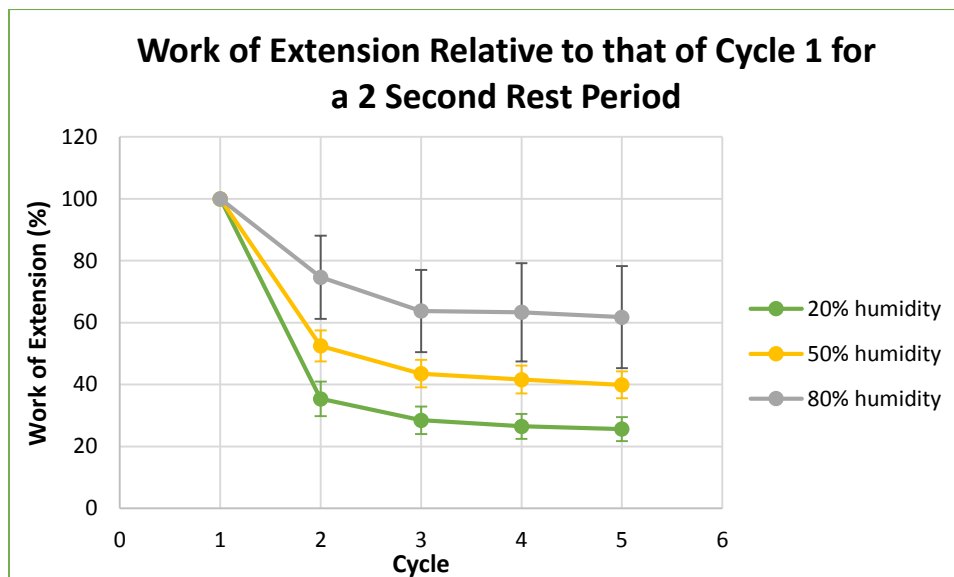


Figure 2: Work of extension data for cyclical tests with a 2 second rest period at 20%, 50%, and 80% humidity. Full recovery was a return to 100% work of extension.

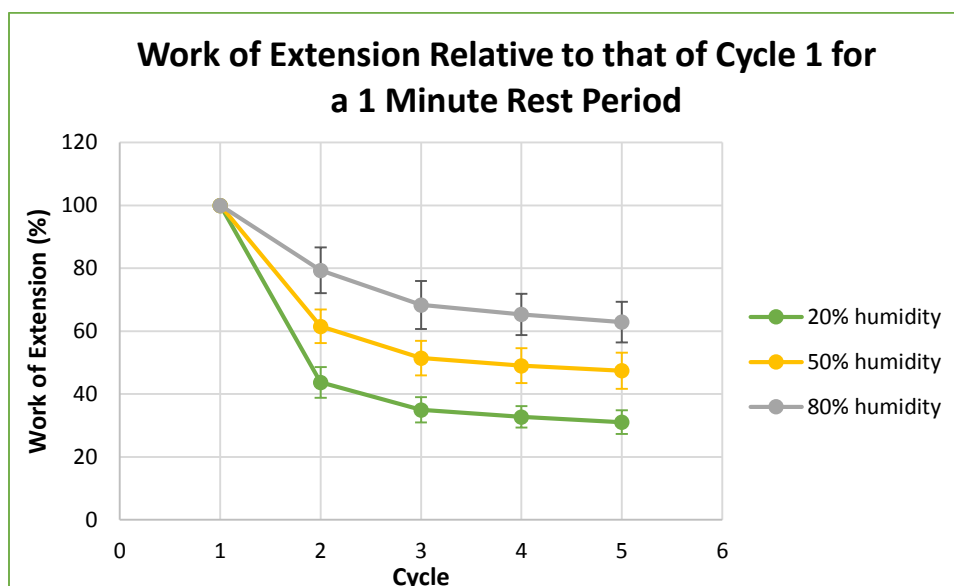


Figure 3: Work of extension data for cyclical tests with a 1 minute rest period at 20%, 50%, and 80% humidity. Full recovery was a return to 100% work of extension.

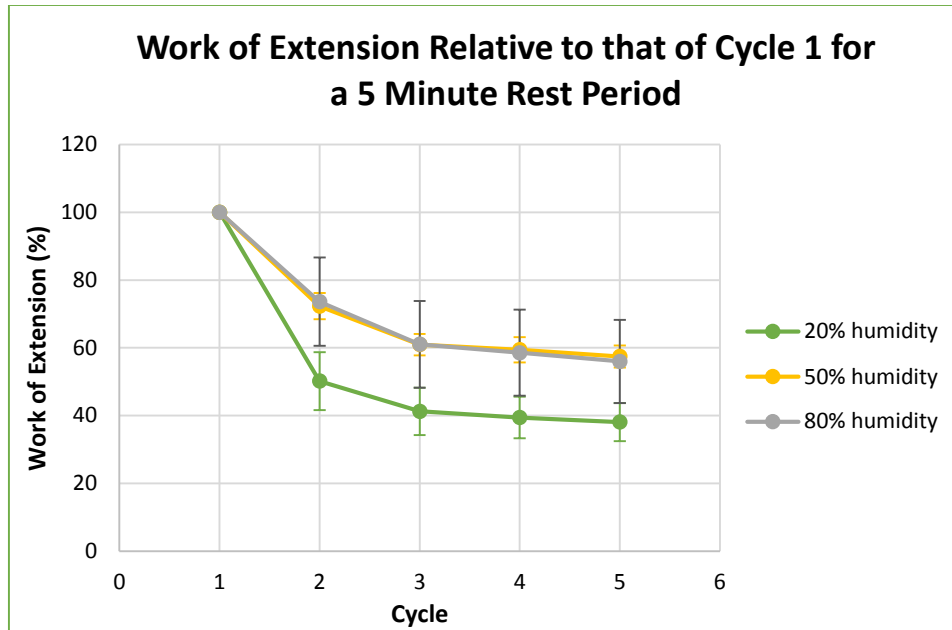


Figure 4: Work of extension data for cyclical tests with a 5 minute rest period at 20%, 50%, and 80% humidity. Full recovery was a return to 100% work of extension.

In Figures 2-4, recovery generally increased with humidity for a given amount of time. Though not confirmed statistically the trend overall, as expected,⁷ appeared to be that work of extension decreased over successive cycles, which signified damage, with a significant drop occurring between cycles 1 and 2. However, the higher the humidity was, the less drastic the drop between cycles 1 and 2 appeared to be. It was shown statistically that as humidity increased, a greater percentage of the original work of extension was required to extend the sample during successive cycles than if the test had been done at lower humidity for all comparisons but 1. The 20% humidity samples, though only experiencing a maximum true stain of 60%, still experienced the least recovery. Again, had those samples also been strained to 80% true strain as was done for the higher humidity samples, the damage was expected to have been even greater.

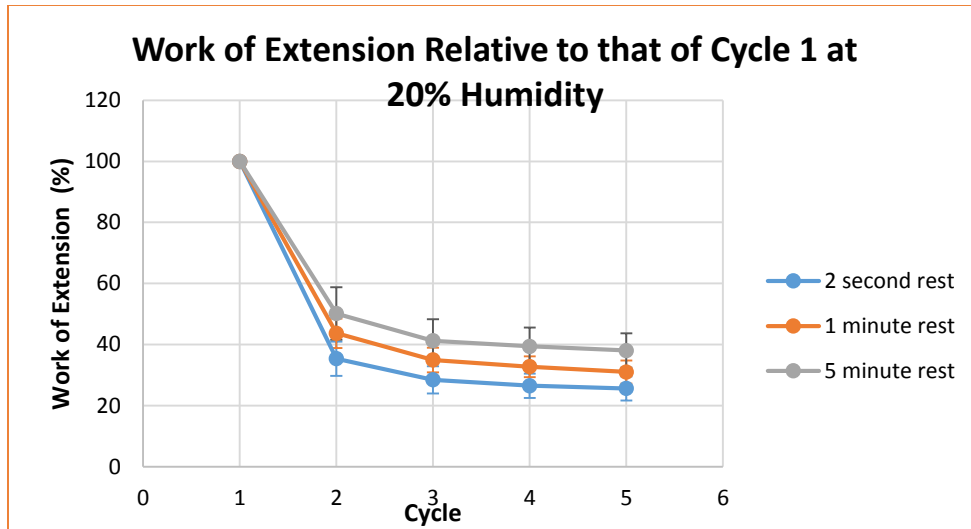


Figure 5: Work of extension data for cyclical tests conducted at 20% humidity with rest periods of 2 seconds, 1 minute, and 5 minutes. Full recovery was a return to 100% work of extension.

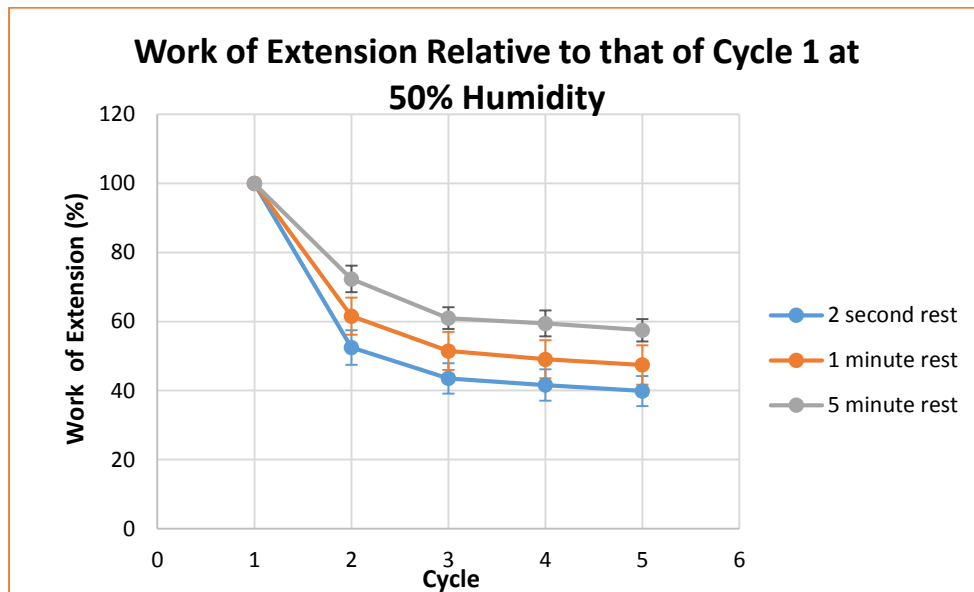


Figure 6: Work of extension data for cyclical tests conducted at 50% humidity with rest periods of 2 seconds, 1 minute, and 5 minutes. Full recovery was a return to 100% work of extension.

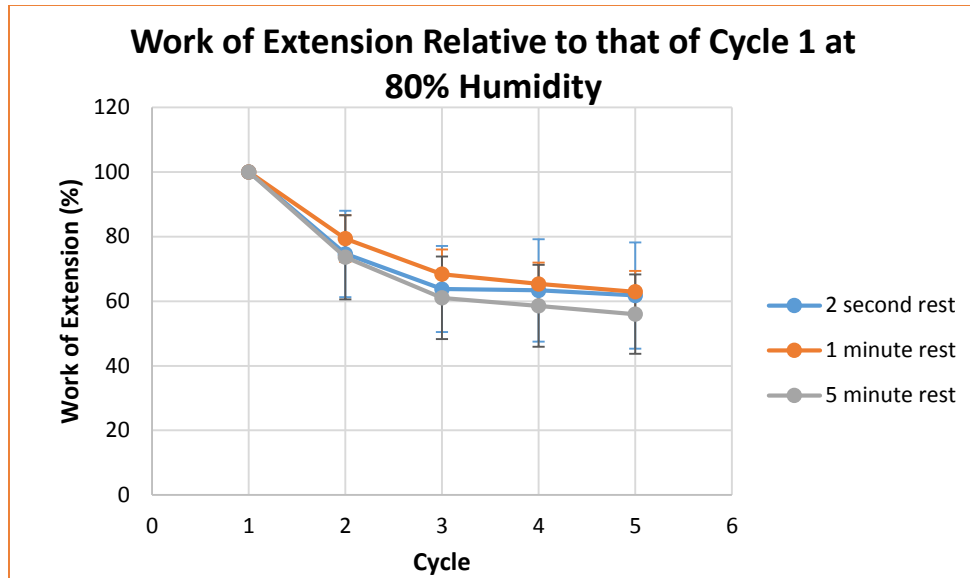


Figure 7: Work of extension data for cyclical tests conducted at 80% humidity with rest periods of 2 seconds, 1 minute, and 5 minutes. Full recovery was a return to 100% work of extension.

Based on Figures 5-7, an overarching trend in recovery related to rest period length could not be identified with certainty. No correspondence between rest period length and extent of recovery was observable at 80% humidity. However, several observations suggested greater recovery at longer rest periods for 20% and 50% humidity. At 50% humidity, recovery clearly increased every time the rest period lengthened. At 20% humidity, this correspondence was seen between the 5 minute rest period and 2 second rest period samples, but not between other rest period pairs. Thus, the data indicated that recovery increased with rest period length only under certain conditions of humidity, more so at intermediate humidity and less so or not at all at more extreme humidity levels, indicating that humidity rather than time was the dominant healing factor for the ranges of humidity and rest period used.

Greater recovery was considered to be a smaller change in hysteresis. For the sake of comparison, hysteresis relative to work of extension was calculated as a percentage of the original hysteresis value of cycle 1. Figure 8 shows the equation used to calculate hysteresis values and explains the variables of that equation. Figures 9-11 show hysteresis for rest periods of 2 seconds, 1 minute, and 5 minutes, respectively, with each figure containing values for each humidity. Figures 12-14 show hysteresis values for 20%, 50%, and 80% humidity, respectively, with each figure containing values for all rest periods. If desired, the P-values for comparisons between hysteresis data of a particular cycle may be viewed in Table S4 in Appendix II.

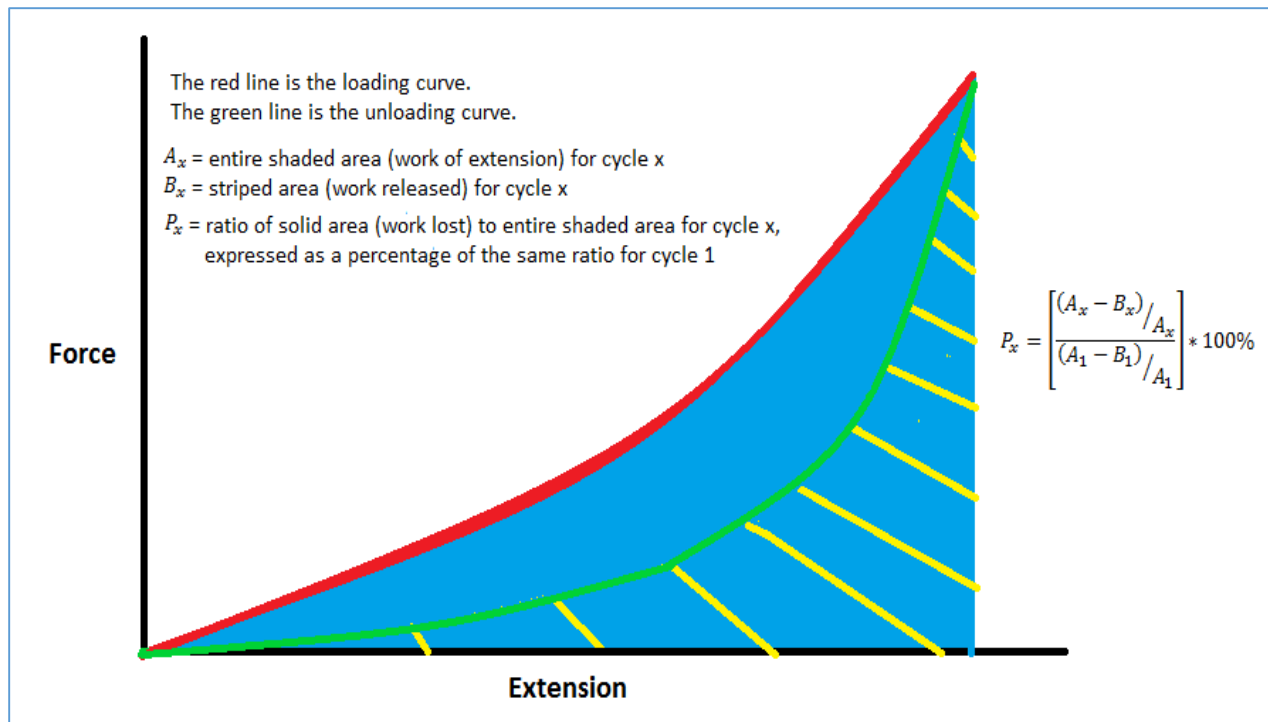


Figure 8: Schematic of a sample load vs. extension curve for a single loading cycle and the formula used to calculate hysteresis for cycle x , P_x .

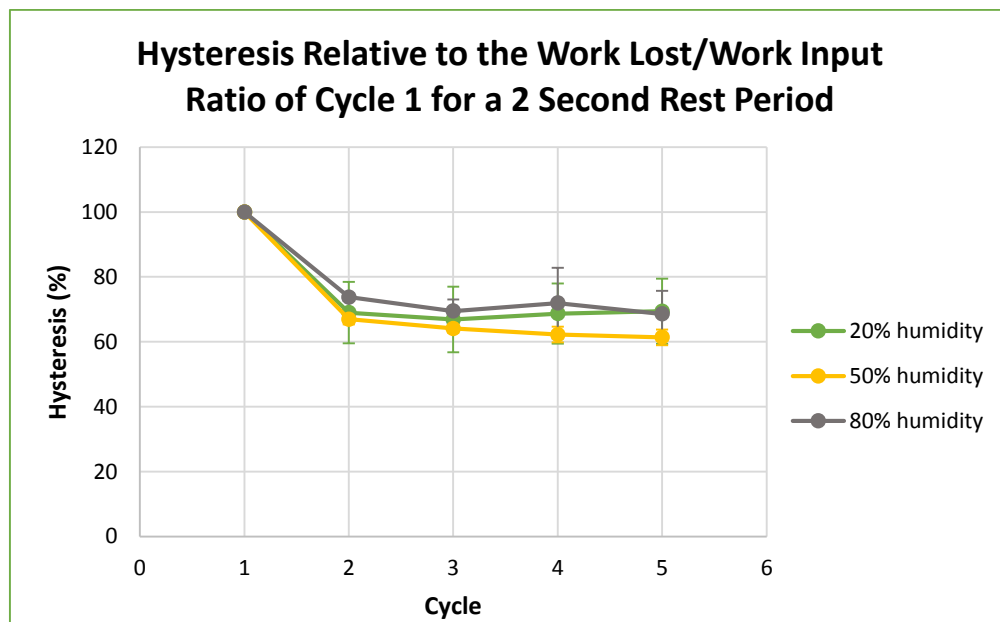


Figure 9: Hysteresis data for cyclical tests with a 2 second rest period at 20%, 50%, and 80% humidity. Full recovery was a return to 100% hysteresis.

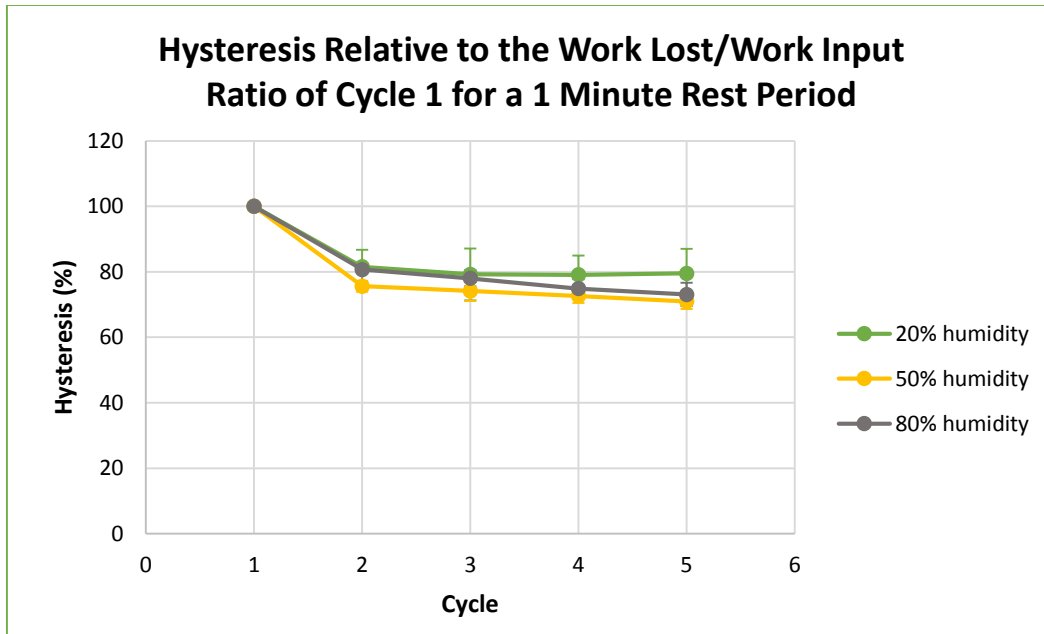


Figure 10: Hysteresis data for cyclical tests with a 1 minute rest period at 20%, 50%, and 80% humidity. Full recovery was a return to 100% hysteresis.

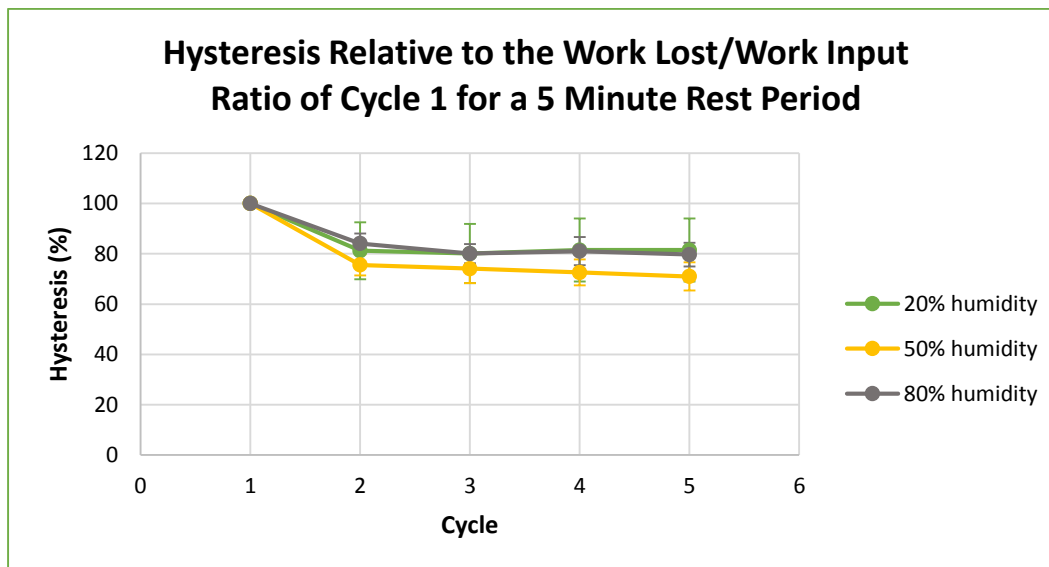


Figure 11: Hysteresis data for cyclical tests with a 5 minute rest period at 20%, 50%, and 80% humidity. Full recovery was a return to 100% hysteresis.

Visually in Figures 9-11, there appeared to be a general drop in hysteresis between cycles 1 and 2 followed by little change in hysteresis values. Thus, the most damage appeared to be incurred during the cycle 1 extension, though statistical tests were not performed to confirm this. However, no humidity-related trends could be established. Had the samples at 20% humidity been extended to the

same strain, a humidity-related trend may have appeared; but despite equal extensions, no trend appeared between the 50% and 80% humidity samples, which may have suggested that hysteresis is humidity independent under the experimental conditions for these samples.

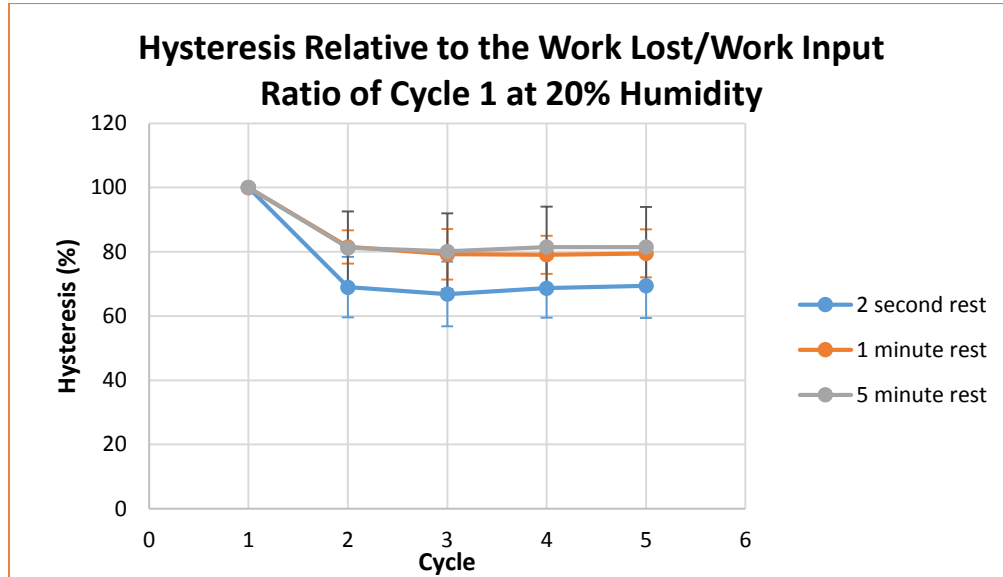


Figure 12: Hysteresis data for cyclical tests conducted at 20% humidity with rest periods of 2 seconds, 1 minute, and 5 minutes. Full recovery was a return to 100% hysteresis.

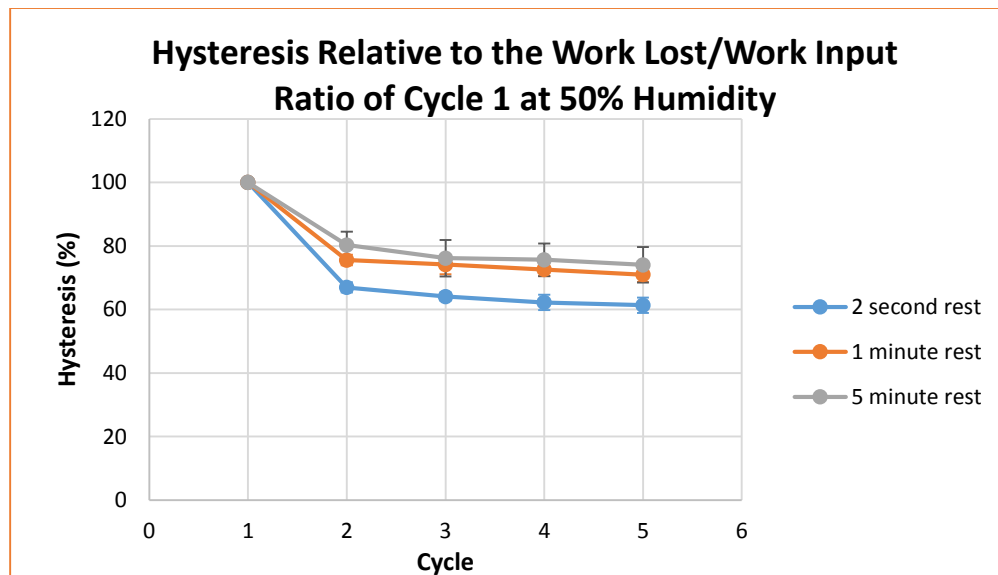


Figure 13: Hysteresis data for cyclical tests conducted at 50% humidity with rest periods of 2 seconds, 1 minute, and 5 minutes. Full recovery was a return to 100% hysteresis.

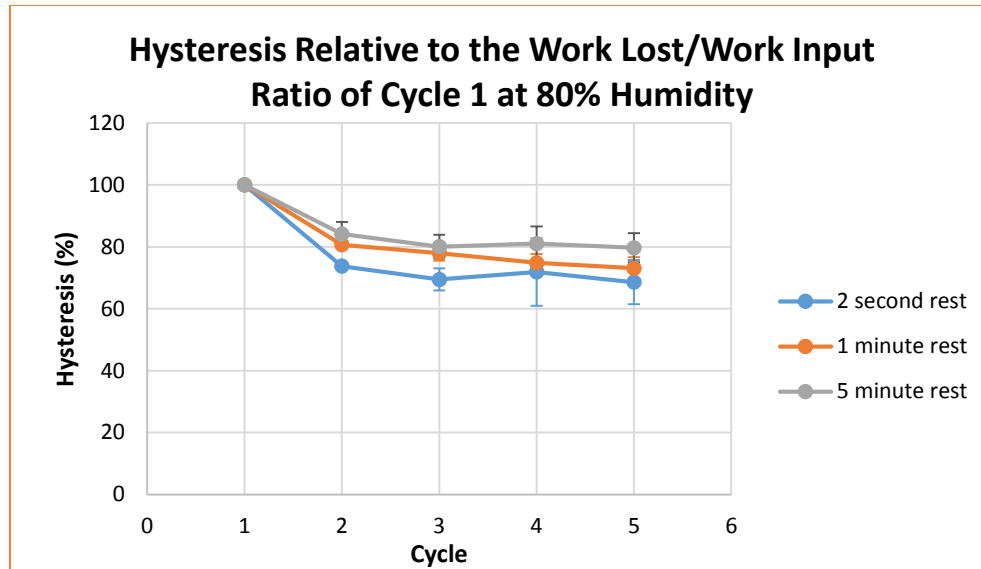


Figure 14: Hysteresis data for cyclical tests conducted at 80% humidity with rest periods of 2 seconds, 1 minute, and 5 minutes. Full recovery was a return to 100% hysteresis.

From Figures 12-14, it was not possible to establish a recovery trend in terms of hysteresis related to rest period length. The 2 second rest period samples at 50% humidity experienced less recovery than the samples with longer rest periods, but no other relationships could be established at this or any other humidity. In Figure 12, the average hysteresis values for the 2 second rest period samples appeared visibly displaced below those for longer rest periods, but there was no significant statistical difference because of variation. The slight indication of greater recovery for longer rest periods only at 50% humidity, in accordance with the work of extension data, may have indicated that this trend is most prominent at intermediate humidity but less prominent or non-existent at more extreme humidity levels.

MICROSCOPE WORK

Since each sample was a pair of silk threads, the effective diameter of a sample was considered to be the diameter of a hypothetical thread whose cross-sectional area equaled the sum of the cross-sectional areas of the actual thread pair. Microscope images of silk samples were not very clear overall. Thus, diameter measurements and calculated effective diameters were approximates. In general, the diameter of a single silk strand measured between 1 μm and 4 μm ; and the effective diameter of a sample, consisting of a silk strand pair, was between 1 μm and 5 μm .

CONCLUSIONS

Cyclical test results indicated that recovery in viscid silk increases with humidity. Cyclical test results also indicated that recovery increases with rest period length under certain humidity conditions, namely intermediate humidity; but this trend was less apparent or did not appear at all at more extreme humidity levels for the rest periods utilized in this research. Because of this, humidity, rather than time, was shown to be the more dominant healing factor in these tests. Hysteresis was relatively unaffected in these tests compared to work of extension, which demonstrated work of extension to be the better

indicator of the relative extent of damage and recovery. Possibilities for further query include increasing the humidity and rest period length to determine if full recovery can be achieved by the methods used in this research, as well as performing similar tests to identify recovery trends with respect to other variables, such as temperature or extension rate.

MATERIALS AND METHODS

CARE OF SPIDERS

Female spiders of the species *Larinioides cornutus* were collected after sundown and housed in individual cages which were approximately 40.6 cm by 40.6 cm by 10.8 cm. The spiders were kept in a greenhouse when silk was not being collected that day. Spiders were normally misted once daily during the work week. Spiders were fed 1 large/medium or 2 small crickets about twice weekly.

SAMPLE COLLECTION

GENERAL

All samples were of pristine capture spiral silk collected from webs made the night before. No threads that appeared damaged or stuck together were collected. If any major ampullate silk threads were broken, any viscid silk threads attached to them were not collected, since the broken support threads caused the attached viscid silk threads to no longer be at native tension. However, viscid silk samples were still collected if they had been stretched slightly beyond native tension due to reduction of web structural stability caused by the breakage of other nearby viscid lines. Samples were not collected from webs that contained crickets or that had been misted for that day. Care was taken to make note of which samples came from which spiders.

TENSILE AND CYCLICAL TEST SAMPLES

Samples were collected on cards of black construction paper which were approximately 2.5 cm by 4.6 cm (the size of a microscope slide), each with a square hole about 12.58 mm wide at one end. A small amount of Elmer's® washable school glue was placed on opposite sides of the square hole. The card was gently touched to the desired capture spiral thread, and a soldering iron was used to cut the ends of the thread. Care was taken to stretch the silk as little as possible during collection and to orient the samples so that they were as parallel as possible to the sides of the holes in the cards.

MICROSCOPE SAMPLES

In order to determine silk strand diameter, capture spiral silk samples were also collected on microscope slides. The slides were touched to the desired viscid silk strands, which became attached to the slides by merit of their own natural glue; and a soldering iron was used to cut the ends. These samples were used to determine the diameter of other samples from the same thread or from threads which were up to two sections directly clockwise or counterclockwise in the web. For example, in Figure 15, the green thread can only be used as a microscope sample to determine the diameter of other samples taken from itself and from the four yellow threads.

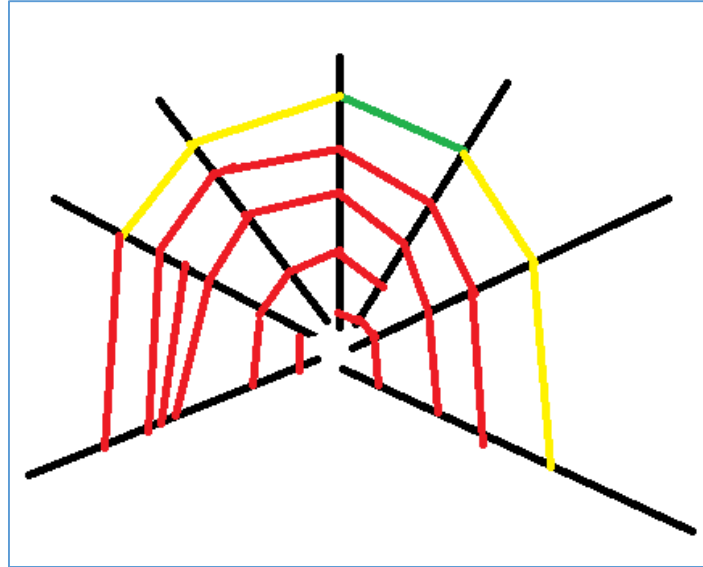


Figure 15: Schematic showing which sections of the web each microscope sample applies to. The green strand is the one from which the microscope sample is taken. This sample can be used to measure the diameter of other samples from the same thread or of samples from the yellow threads, but not of samples from the red threads.

TENSILE AND CYCLICAL TESTS

GENERAL

All tests were performed using a Nano Bionix® UTM tensile tester, and data was analyzed and calculated using TestWorks® 4 software and Microsoft® Excel. The tensile tester was set on an air table which was kept afloat using compressed nitrogen. Temperature was considered to be the room temperature, as measured by an alcohol in-glass thermometer, for all tests.

A small clear plastic chamber was placed on top of the tensile tester around the silk sample to gain the desired humidity. Humidity was controlled using a flow of compressed nitrogen through a Watlow® humidity controller. A flow of air through a bottle of tap water was also used as needed to reach higher humidities. If needed, small plastic weigh boats with lukewarm deionized water and cotton were used to boost humidity inside the chamber. Just before testing, silk samples were allowed to sit undisturbed inside the humidity chamber for two minutes in order to adjust to the desired humidity. Occasionally, the humidity chamber was not used if the ambient humidity was near the desired humidity. Each type of test was done at three different relative humidities: 20%, 50%, and 80%, within about 6% deviation.

For all tests, the nominal gage length (original sample length) was taken to be 12.58 mm, the width of the holes on the sample-collecting cards (even if the samples were not oriented parallel to the sides of the square holes, though parallel orientation was the goal). All tests had an extension rate of 1 mm/s. Silk was not relaxed before testing, but was tested at its native tension.

TENSILE TESTS

Before conducting tensile tests on silk samples each day, several tests were performed without silk in which the machine was allowed to extend until just before hitting its stopper (which had been previously set) so that error in the load could be gauged. For these tests, the zero load and zero displacement reference markers were placed at the first data point.

Two silk samples per spider per humidity setting were extended to breakage. Zero load and zero displacement reference markers were placed on top of each other at the point where true strain began to noticeably increase when the true strain vs. time curve was zoomed in to ± 0.010 mm/mm on the y-axis. The break index reference marker (marker for where the sample snapped) was placed at the point where the load on the specimen began to drop rapidly to zero. True and engineering values of extensibility were determined for each test, and the averages and standard deviations of these two extensibilities for each humidity setting were calculated.

CYCLICAL TESTS

No error-gauging tests were conducted.

Hysteresis tests consisted of six cycles, though only data associated with the first five was analyzed. For each cycle, the silk was extended the maximum strain and returned to the zero extension length after a rest period of about 0.250 s at the maximum strain. The rest period after each full cycle at the zero extension length was set to 2 seconds, 1 minute, and 5 minutes for different tests. (The machine never overshot the return to zero extension by more than 7%.) The maximum strain was 80% true strain for tests at 80% and 50% relative humidity, and was 60% true strain for tests at 20% relative humidity. (These maximum strain values induced loads that were significantly larger than the machine noise without breaking most of the threads.) Samples from three spiders were used. Two silk samples per spider per humidity setting/recovery time combination were tested. Zero load and zero displacement reference markers were placed on top of each other where true strain began to increase rapidly in the true strain vs. time curve. However, because of drift in the load reading, the zero load reference marker was moved to the beginning and end cycle markers for each cycle in order to determine work of extension and work of relaxation, respectively. Markers for the beginning and end of cycles were placed at the same level of true strain as the zero displacement reference marker. Markers for the middle of cycles were placed accordingly. Hysteresis, work of extension, and work of relaxation were calculated. Two-tailed P values from unpaired *t* tests conducted online at <http://www.graphpad.com/quickcalcs/ttest1/> were examined to determine if difference in the data was statistically significant. A P value less than 0.05 was considered to indicate significant statistical difference. If a sample broke at or just before reaching the maximum strain, the work to breakage was considered the work of extension for that cycle.

MICROSCOPE WORK

Samples taken previously on microscope slides were examined at 100x magnification using a Leica® DM LB2 microscope. Image-Pro® 6.2 software was used to take pictures of the silk and measure the diameter of a single silk thread. If necessary, a dropper was used to add water to the slide to facilitate separation of the thread pairs. Excess water was absorbed by/dripped off of the microscope slide and onto a paper towel. Water sometimes caused the silk to move and stretch slightly on the slides, but this

was assumed to not significantly affect silk diameter. Single threads were assumed to be perfectly cylindrical, and both threads in each sample were assumed to have equal diameters. Three single-strand diameter measurements were made per sample. The average and standard deviation for the single-strand diameter and the effective diameter for each sample were calculated. The diameter of a single silk thread (d_1) and the effective diameter of the thread pair (d_e) were related by the following formula, which followed from equating the sum of the circular cross-sectional areas of the thread pair with the formula for the cross-sectional area of the hypothetical thread:

$$d_e = \sqrt{2}d_1 \quad (1)$$

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APPENDIX I

SAFETY CONSIDERATIONS

Care was required when using the heated soldering iron to avoid contact with skin and flammable materials. Care was required to keep fingers, hair, and loose clothing away from movable parts of the Nano Bionix® tensile tester during operation. Bites from spiders used in this research were not known to pose a serious threat. However, to avoid spider bites, care was taken to avoid contact with the spiders using bare hands. No chemically or biologically hazardous materials were used in this study.

APPENDIX II

SUPPLEMENTAL INFORMATION

Spider of Origin	Relative Humidity (%)	True Extensibility	Engineering Extensibility
3	80	1.445	3.240
3	80	1.018	1.768
5a	80	1.163	2.200
5a	80	1.261	2.528
4	80	1.412	3.104
4	80	1.281	2.600
6	80	1.135	2.112
6	80	1.224	2.400
2	80	0.937	1.552
2	80	1.055	1.872
3	50	1.093	1.984
3	50	1.072	1.920
5a	50	1.173	2.232
5a	50	1.138	2.120
4	50	1.000	1.720
4	50	1.200	2.320
6	50	1.296	2.655
6	50	1.265	2.544
2	50	1.030	1.800
2	50	1.038	1.823
3	20	0.570	0.768
3	20	0.717	1.048
5a	20	0.992	1.696
5a	20	0.977	1.656
4	20	0.736	1.088
4	20	0.640	0.896
6	20	0.697	1.007
6	20	0.908	1.480
2	20	0.796	1.217
2	20	0.892	1.440

Table S1: Results for tensile tests on individual silk samples. Only data for samples taken from spiders 2, 3, and 6 were used to construct Table 1 in the main body of this report, since those were the 3 spiders samples for cyclical tests were taken from.

	20% Humidity	50% Humidity	80% Humidity
True Extensibility			
Average	0.793	1.131	1.193
Standard Deviation	0.137	0.10	0.156
Engineering Extensibility			
Average	1.230	2.112	2.338
Standard Deviation	0.3	0.3	0.5

Table S2: Averages and standard deviations of extensibility incorporating data from all the spiders in Table S1.

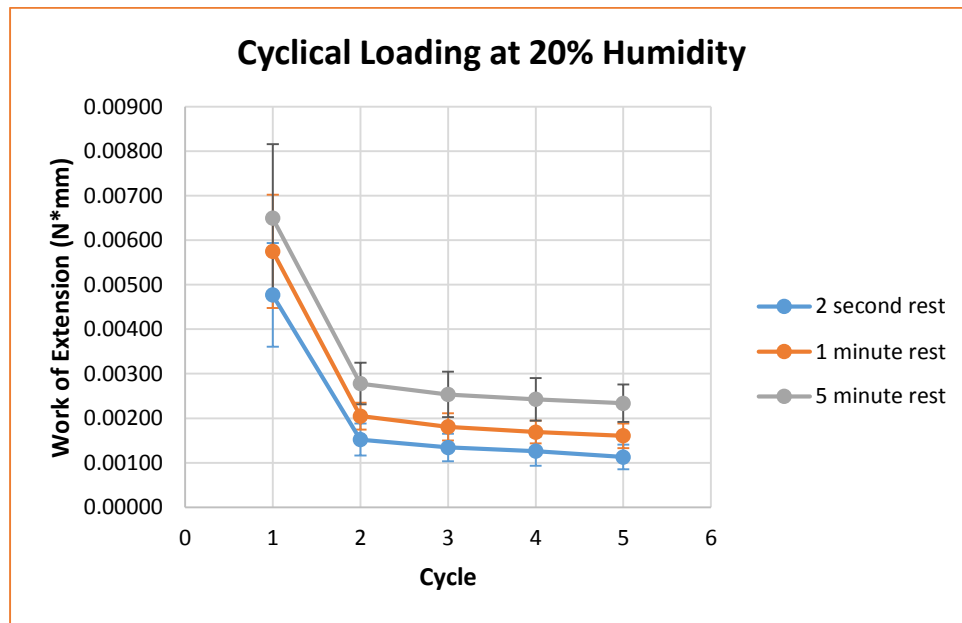


Figure S1: Absolute values of work of extension at 20% humidity for the 3 rest periods.

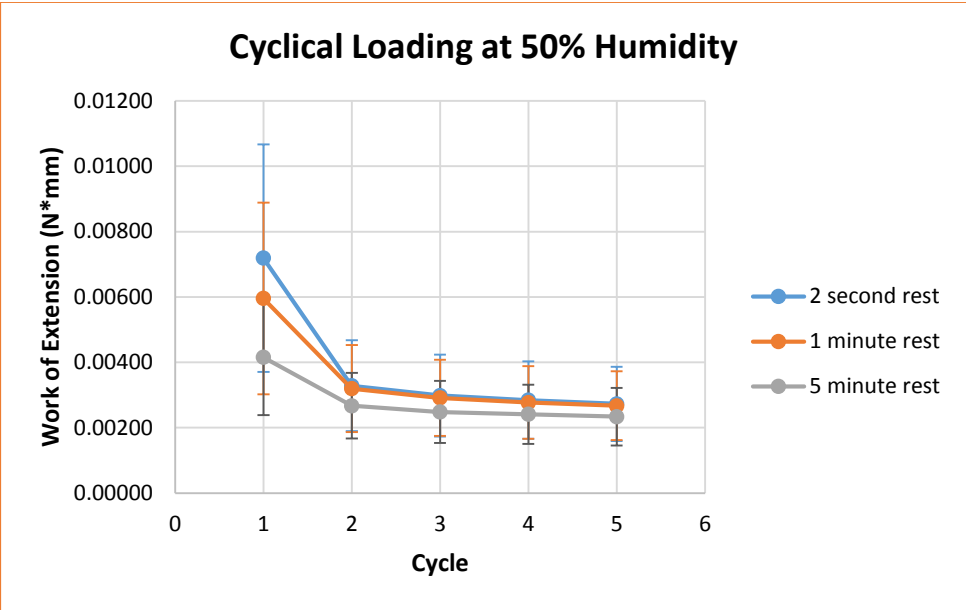


Figure S2: Absolute values of work of extension at 50% humidity for the 3 rest periods.

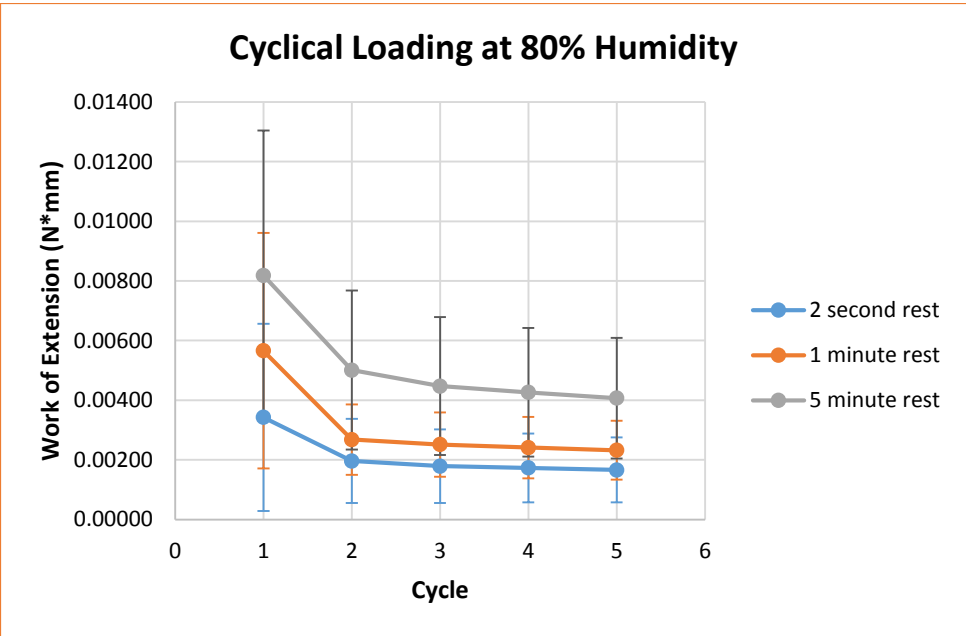


Figure S3: Absolute values of work of extension at 80% humidity for the 3 rest periods.

		P VALUE				
Humidity	Rest Period Comparison	cycle 1	cycle 2	cycle 3	cycle 4	cycle 5
20%	2 s/1 min		0.0778	0.0857	0.0653	0.1333
	2 s/5 min		0.0201	0.0149	0.0079	0.0129
	1 min/5 min		0.2719	0.2002	0.1299	0.1013
50%	2 s/1 min		0.0199	0.0304	0.0419	0.0408
	2 s/5 min		0.0001	<0.0001	0.0001	<0.0001
	1 min/5 min		0.0079	0.0129	0.0100	0.0115
80%	2 s/1 min		0.5939	0.6048	0.8413	0.9110
	2 s/5 min		0.9094	0.7638	0.6266	0.5635
	1 min/5 min		0.4926	0.3863	0.4043	0.3821
Rest Period	Humidity Comparison (%/%)	cycle 1	cycle 2	cycle 3	cycle 4	cycle 5
2 s	20/50		0.0009	0.0007	0.0005	0.0015
	20/80		0.0006	0.0010	0.0020	0.0069
	50/80		0.0079	0.0115	0.0175	0.0199
1 min	20/50		0.0014	0.0017	0.0015	0.0020
	20/80		0.0004	0.0005	0.0003	0.0003
	50/80		0.0040	0.0067	0.0053	0.0073
5 min	20/50		0.0015	0.0009	0.0005	0.0003
	20/80		0.0122	0.0209	0.0210	0.0235
	50/80		0.8485	0.9878	0.8934	0.8178

Table S3: P Values from the *t* Tests for Work of Extension Data. These *t* tests were not conducted for cycle 1 data, since cycle 1 data points had no variation at 100%. The *t* tests were unpaired and the P values were 2-tailed. P values less than 0.05 were considered to indicate significant statistical difference and are shown in green.

		P VALUE				
Humidity	Rest Period Comparison	cycle 1	cycle 2	cycle 3	cycle 4	cycle 5
20%	2 s/1 min		0.0731	0.1199	0.1528	0.2126
	2 s/5 min		0.1366	0.1260	0.1762	0.2100
	1 min/5 min		0.9635	0.9117	0.7621	0.8136
50%	2 s/1 min		<0.0001	<0.0001	<0.0001	<0.0001
	2 s/5 min		0.0001	0.0015	0.0006	0.0014
	1 min/5 min		0.0460	0.5195	0.2567	0.2917
80%	2 s/1 min		<0.0001	0.0088	0.6563	0.3463
	2 s/5 min		0.0006	0.0021	0.1407	0.0209
	1 min/5 min		0.1588	0.4189	0.1058	0.0652
Rest Period	Humidity Comparison (%/%)	cycle 1	cycle 2	cycle 3	cycle 4	cycle 5
2 s	20/50		0.6540	0.5621	0.1814	0.1314
	20/80		0.3428	0.6371	0.6905	0.9052
	50/80		<0.0001	0.0129	0.0880	0.0645
1 min	20/50		0.0469	0.2385	0.0601	0.0478
	20/80		0.7906	0.8086	0.3117	0.2296
	50/80		0.0009	0.0947	0.2321	0.3402
5 min	20/50		0.8865	0.5623	0.4146	0.3124
	20/80		0.6073	0.9937	0.9431	0.7801
	50/80		0.1892	0.2529	0.1708	0.1354

Table S4: P Values from the *t* tests for hysteresis data. These *t* tests were not conducted for cycle 1 data, since cycle 1 data points had no variation at 100%. The *t* tests were unpaired and the P values were 2-tailed. P values less than 0.05 were considered to indicate significant statistical difference and are shown in green.